# Ceramic Capillaries for Use in Microarray Fabrication

Reed A. George*, John Patrick Woolley, and Paul Spellman

Berkeley Drosophila Genome Project (BDGP), Lawrence Berkeley National Laboratory, One Cyclotron Road, Berkeley, CA 94720, USA.

* corresponding author

**Abstract**

We have used ceramic capillary tips generally used in the microelectronics industry for the production of DNA microarrays. The ceramic tips improve the morphology of microarray elements, allow higher element density, and increase printing tip life over the customary slotted stainless steel pins. Ceramic capillaries are less expensive than steel pins and allow printing from 1536-well sample source plates. In this paper, we describe experiments that verify (establish?) printing performance of these ceramic tips, and hybridization experiments that demonstrate that DNA hybridization is unaffected by the choice of tip material.

**Introduction**

Over the past several years, the applications of DNA microarray technology have diversified from the initial gene expression studies in yeast (Schena *et al.* 1995, DeRisi *et al.* 1997) to include the characterization of human cancer tumor tissues (Perou *et al.* 2000), high-throughput SNP genotyping (Hacia *et al.* 1999), and the study of *in vivo* binding of transcription factor proteins (Ren *et al.* 2000). Two very different technical approaches to microarray production have enabled this rapid expansion: synthesis of short oligonucleotide probes directly on glass surfaces (*in situ* synthesis) and the deposition of probes (either PCR products or oligonucleotides) onto glass surfaces using either metal pins or modified ink jet systems (spotted arrays). A low-cost robotic system developed at Stanford University for producing spotted arrays has found application in many laboratories (Schena *et al.* 1995, Bowtell *et al.* 1999). This system uses stainless steel printing tips, similar in design to a fountain pen, to aspirate and deposit DNA onto glass microscope slides. Through repeated cycles of sample aspiration, deposition onto a series of slides, and cleaning of the printing tips, high-density microarrays are produced.

We have developed a microarray production method that uses cylindrical ceramic capillary tips instead of stainless steel tips. The capillaries are adapted from microelectronics applications, where they are typically used for bonding high-density interconnections (wires less than .001" in diameter) during the assembly of integrated circuit packages. The capillaries are manufactured to very tight tolerances, and are available commercially. Our data demonstrate that the ceramic tips improve the consistency of deposit morphology, resist deformation over long-term use, cost less, and offer the potential for significant improvement in deposit density.

**Results**

In order to evaluate the morphology of deposits spotted with ceramic tips, a solution of DNA amplified using PCR with nucleotides labeled with cy3 fluorescent dye was printed onto standard microscope slides coated with poly-L-lysine (Schena *et al*. 1995). After spotting, the diameters of the deposits were measured using a scanning fluorescence imager. Figure 1 shows a histogram comparing the distribution of diameters of spots deposited using stainless steel and ceramic tips (n = 1024 for each tip type). The ceramic capillaries yield more reproducible results, with a standard deviation in spot diameter among spots produced by a single tip of less than 4 microns, and a standard deviation in spot diameter between tips of 10 microns (data not shown).

Similar experiments were conducted to assess the range of deposit diameters that can be applied using various sizes of ceramic tips. Figure 2A shows a selection of ceramic tip designs, with tip diameters ranging from 50 microns to 178 microns. Table 1 shows the spot diameters that were applied using various ceramic tip sizes, ranging from approximately 60 to 130 microns in diameter. Spot size is proportional to the outer diameter of the capillary, suggesting that further reductions in element size can be obtained by the production of smaller capillaries. In order to evaluate the potential for increased spot density using ceramic tips, the smallest available standard ceramic tip was used to print labeled DNA at 100 micron spacing (figure 3), yielding a two-fold higher density than we have been able to achieve with stainless steel tips.

The ceramic tips also exhibit superior resistance to deformation after repeated use. Figure 2B illustrates deformation of stainless steel tips as a result of array printing (approximately 100,000 spots deposited), in contrast to ceramic tips, which show no visible wear. The stainless steel tips tend to deform over time to an extent that is dependent upon the contact force experienced during microarray printing. This deformation leads to variation in spot diameter and morphology over time, and from tip to tip. The ceramic tips do not deform, and therefore provide increased consistency in spot diameter and morphology.

In order to demonstrate that probes deposited with ceramic tips hybridize similarly to those deposited with standard metal tips, a hybridization experiment was conducted. A series of 1 kb PCR products, covering 2.8 Mb of the *Adh* region of *Drosophila melanogaster*, were used to manufacture a microarray. Genomic DNA spiked with known amounts of DNA from BACs known to be included in this region served as probe. Probe DNA was amplified with random hexamers and Cy3-labeled nucleotides. Purified *Drosophila* genomic DNA was labeled with Cy5-labeled nucleotides and served as the reference sample. After hybridization, the microarrays were scanned in a fluorescent scanning imager. Deposits corresponding to the regions covered by the "spiked" BAC clones were higher in signal in the Cy3 channel, correctly indicating increased abundance of DNA from these regions in the test sample. Results from microarrays produced using stainless steel and ceramic printing tips correlate well, indicating no systematic bias between arrays produced using the two types of pins. Figure 4 shows the data from these two experiments. Each data point represents a single deposit on the microarray, and the log base 2 ratio of cy3 / cy5 signal is plotted for the arrays produced with metal and ceramic tips, on the X and Y axes, respectively. The solid diagonal line represents exact correlation.

The time required to print microarrays is inversely proportional to the number of tips that can be used simultaneously in a given area. Traditionally we (and others) have manufactured microarrays using 16, 32 or 48 tips, with a tip density of about 5 tips / $cm^2$ (printed from 384-well microtiter plates). We have successfully printed microarrays using DNA samples (100 ng/µl sheared herring sperm DNA) arrayed in 1536-well source plates, potentially allowing a tip density of 20 tips /$cm^2$. Hybridization experiments at this density have not yet been performed.

**Discussion**

The two-fluor hybridization approach typically used in microarray experiments minimizes the effects of spot variations on experimental results; however, highly repeatable printing is preferable. Consistent, repeatable element size and morphology allow a more straightforward comparison of experimental results, and improve the ease and accuracy of image analysis. The ceramic tips provide more consistent deposit morphology and diameter. This is due in part to their resistance to deformation over time. The extent of deformation of stainless steel tips is a function of printing parameters, including spring force, vertical printing speed, and co-planarity of the print head to the microarray substrate. As a result, each stainless steel tip deforms differently, yielding variation in the contact area of the tip to the microarray substrate.

Because of the wide range of designs available, ceramic tips also offer significantly more flexibility than stainless steel tips. The mean diameter of target spots can be controlled by selecting the appropriate ceramic tip. This is an obvious advantage in terms of increasing the density of spots in microarray experiments by applying the smallest possible deposits. Initial experiments have shown the capability of printing deposits at 100µ spacing, allowing at least a two-fold increase in spot density. Our results suggest that array element spacing could be reduced to 75µm or less (yielding nearly a four-fold increase in density over arrays printed at 135µm spacing). However, it may be advantageous to select tips that apply larger deposits in some applications, for example in those where the quantity of immobilized probe molecules is limiting.

Implementation of ceramic tips is straightforward, and drawings for the tooling used to adapt the tips to existing equipment are available. We have found that the ceramic tips are more sensitive to inaccuracies in leveling of the printing tip holder to the arrayer slide platter. As a result, the tip

holder must be carefully aligned to prevent an increase in deposition failure. Another issue discovered early in the implementation was that ceramic tips are more difficult to dry after cleaning, and if they are not completely dry, the next DNA samples do not aspirate completely. We found that it was possible to dry the tips completely by adding a forced-air drying reservoir. Additionally, recent experiments have shown that cleaning the tips in warm water aids in preventing tip clogs. Figure 5 shows a picture of the tip assembly. Drawings for the reservoir are also available.

Finally, the ability to easily print samples from 1536-well source plates will significantly reduce the amount of labor required for microarray production. Ceramic tips can aspirate liquids without being completely submerged. This, along with the tapered design of the tips, provides for easier alignment, as the tapered tip is much smaller in diameter than the source plate well. In the case of the stainless steel tips, the tapered portion is significantly shorter than that of the ceramic tips. This limits the tolerance for misalignment of the pin to the source plate well, because the diameter of the pin at the top of the taper is much closer in size to the diameter of the source plate well.

## Methods

### Capillary Availability and Tip Holder Assembly

Ceramic tips were purchased from K&S MicroSwiss (Willow Grove, Pennsylvania). They are significantly lower in cost than commercial stainless steel tips. Design drawings for the tip holder and dryer assembly are available through licensing (at no charge for academic organizations) from the Lawrence Berkeley National Laboratory, Berkeley, California.

### Fluorescent Labeled PCR Products for Deposit Morphology and Diameter Tests

Labeled PCR products were produced using a standard protocol (http://genome-www.stanford.EDU/pbrown/) with template DNA from a BAC clone (BACR48E02) and a pair of unique primers. The labeled PCR product was then precipitated with a standard ethanol precipitation protocol and resuspended in 30µl of deionized water. Prior to spotting onto microarray substrates, the products were diluted to 100 ng/µl in 3X SSC.

### Microarray Hybridization Experiments

Microarrays representing the *Adh* region of the *Drosophila melanogaster* genome were manufactured. The elements of the array correspond to non-overlapping PCR fragments (made by standard protocols using BAC DNA as template) of about one kb spanning the region. Fluorescent labeled probes for hybridization were produced by Klenow labeling (Pollack *et al*. 1999) of either genomic DNA, or genomic DNA with three individual BACs added at different ratios. All samples were sheared by sonication to about 300-800 bp. The BACs added, with concentration over genomic in parentheses, were BACR18J08 (5X), BACR48E02 (25X), BACR16P12 (125X).

Protocols for poly-L-lysine coating, probe preparation and arraying, array post-processing, DNA labeling, hybridization, washing, and scanning have been described elsewhere, and detailed protocols are available at (http://genome-www.stanford.EDU/pbrown/ and in (Eisen *et al*. 1999)).

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**Table 1** Mean Spot Diameters for Various Ceramic Tip Sizes. Microarrays were fabricated using ceramic capillaries of various sizes. The diameter of the tip and the resulting spot diameter are indicated.

| Ceramic Tip Diameter (microns) | Mean Spot Diameter (microns) |
|---|---|
| 50 | 56 |
| 83 | 99 |
| 109 | 108 |
| 132 | 130 |

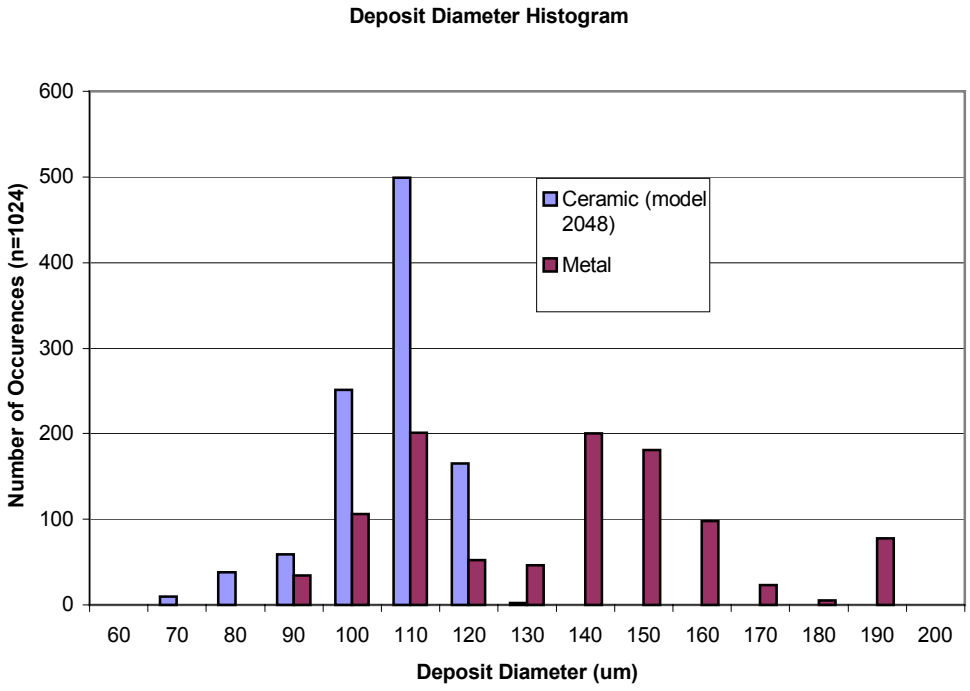
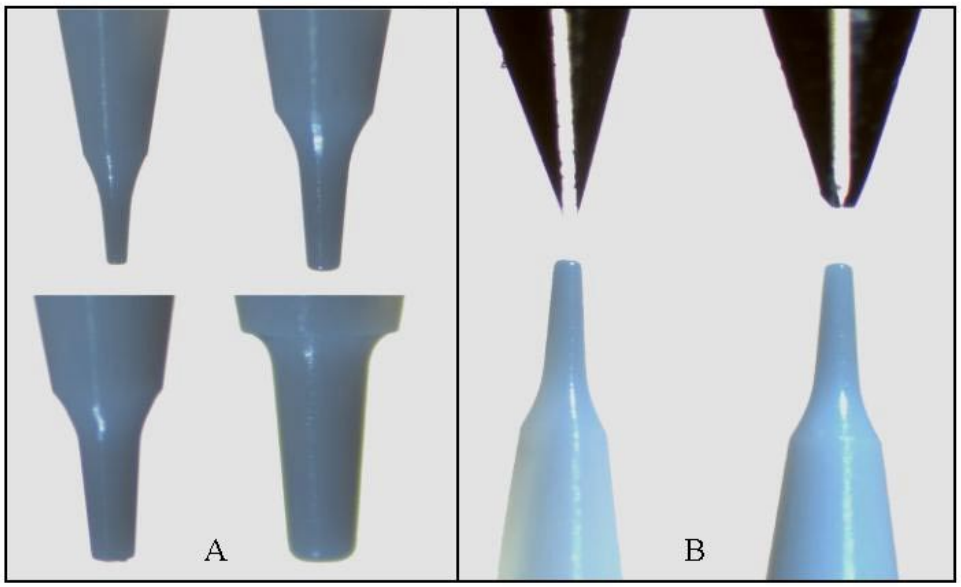



**Figure** 1 Deposit Diameter Distributions for Ceramic and Stainless Steel Printing Pins.

Microarrays were printed using either stainless steel or ceramic tips. The histogram of the sizes

of elements produced by each type of tip is shown.




Fi**gure 2** Images of Tips**.** Panel A shows ceramic capillaries with cross sectional diameters of 50, 83, 109, and 132 microns, all of which have been successfully used to fabricate microarrays. Panel B shows stainless steel slotted tips and ceramic capillaries before (on the left) and after (on the right) printing tens of thousands of microarray elements. The stainless steel tips are considerably deformed, but the ceramic capillaries are intact.

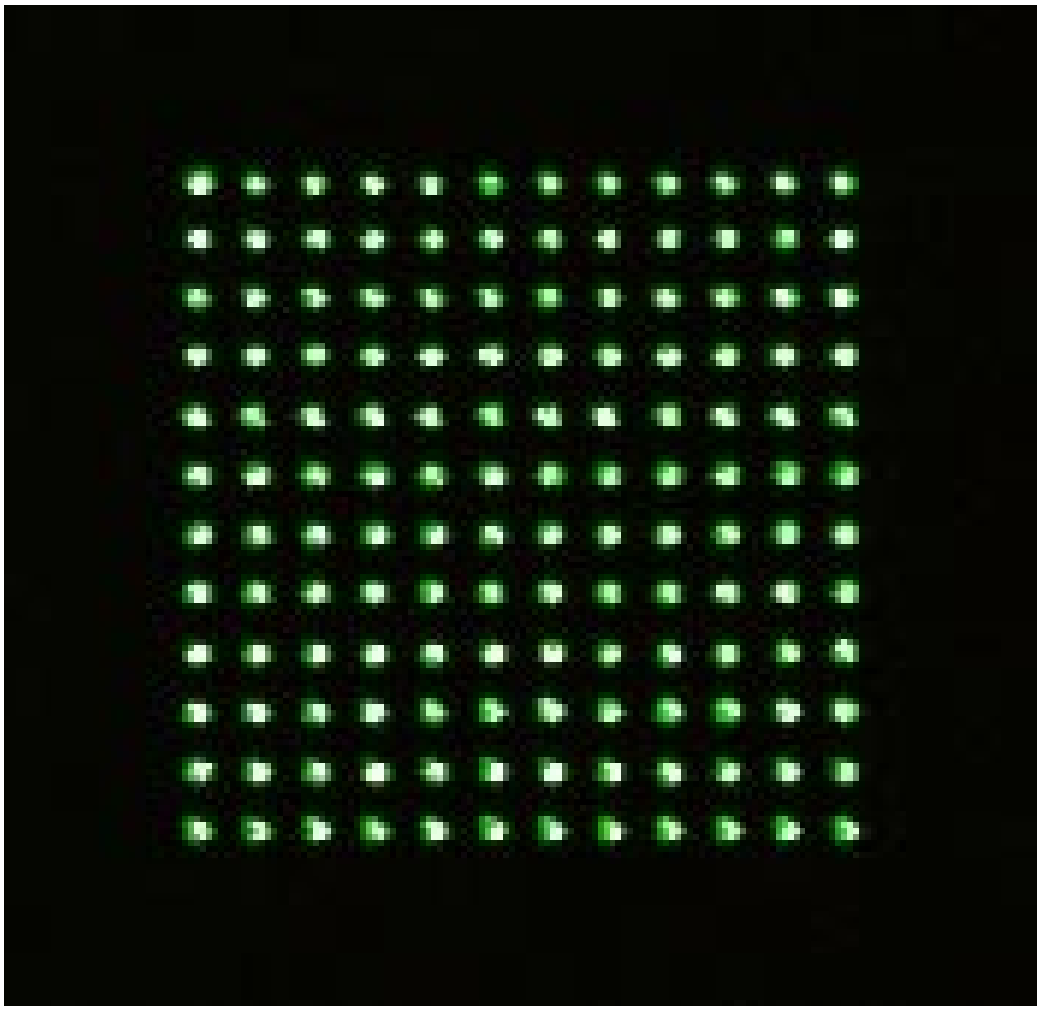


**Figure 3** Fluorescent Image of Labeled DNA Deposited at 100 Micron Spacing.

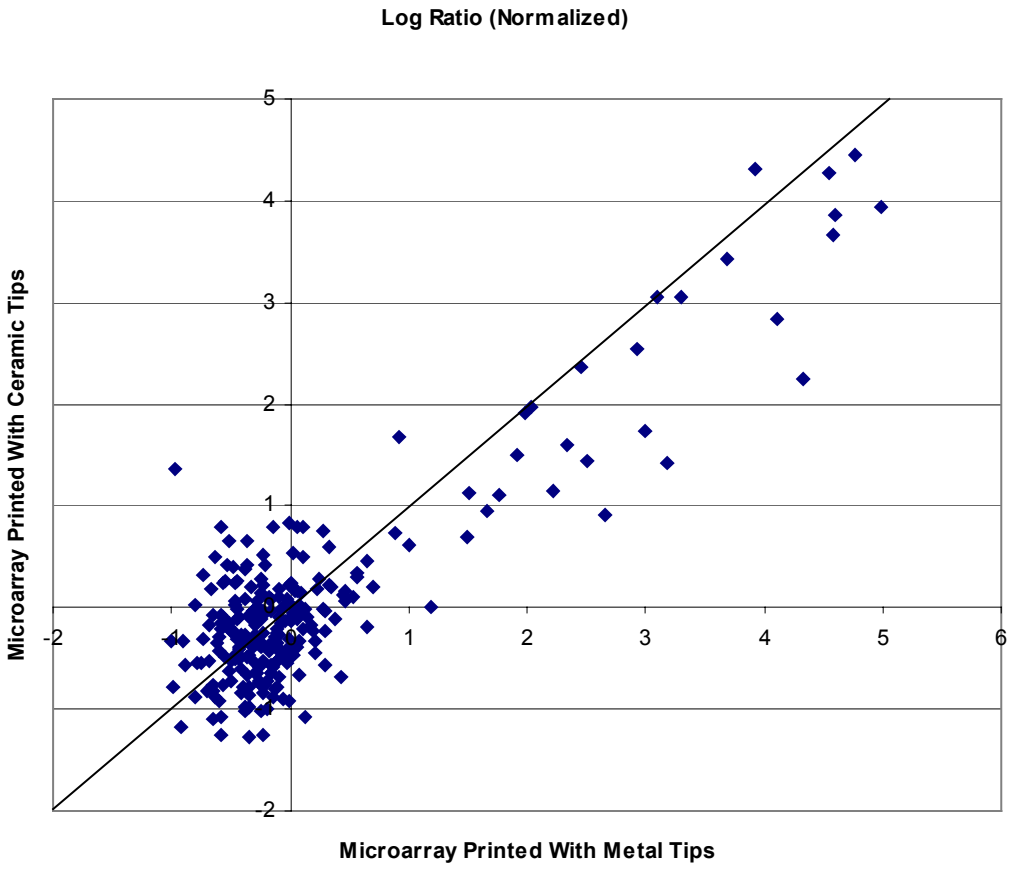



**Figure 4** Comparison of Log Ratio of Fluorescent Hybridization Signal Between Microarrays Printed with Metal and Ceramic Printing Tips. Microarrays were generated (see text) using both steel and ceramic tips and hybridized with genomic DNA spiked with known BACs. The ratio for each element is plotted for each type of array. No significant bias exists between the two data sets.

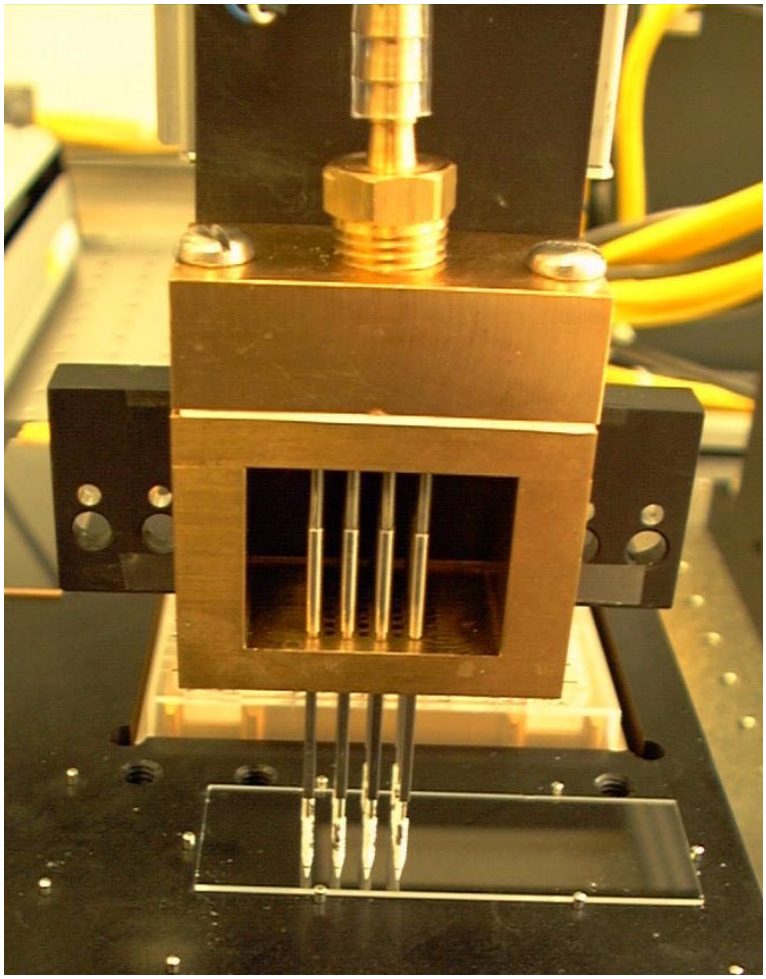


Figure 5. The fully assembled print head. Capillaries (white) are mounted on hollow shafts held in place by a brass print head. The head is covered by a forced air manifold that forces air through the shaft and out of the capillary.